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Assaying and explaining multidrug resistance in Gram-negative bacteria

Validation of methods for enrichment of ESBL and AmpC producing *E. coli* in meat and cecal samples

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Objectives.

It has been decided by the European Commission (Decision 2013/652/EU) to initiate a mandatory pan-European specific monitoring of extended-spectrum (ESBL) and AmpC beta-lactamase producing *Escherichia coli* in meat and cecal samples of both porcine and bovine origin. The isolation method to be used should ensure high detection levels of *E. coli* producing ESBL and AmpC enzymes most commonly found in animal production as well as carbapenemases most commonly found in human infections. Thus, to detect these isolates in the mentioned matrices, different methods were validated.

Methods.

E. coli isolates producing the following ESBL/AmpC/carbapenemase were included in the study: CTX-M-1, SHV-12, TEM-52, CMY-2, VIM-1, KPC-2, NDM-1 and OXA-48. Bacterial suspensions were added (spiked) to the matrices in concentrations of 0.1 (only meat samples), 1, 10, 100 and 1000 CFU/gram matrix sample. All methods included a pre-enrichment step in either Buffered Peptone Water (BPW) and incubation over night at 37°C or MacConkey broth incubated over night at 44°C with or without addition of a third generation cephalosporin and subsequently semi-quantitative plating on MacConkey agar supplemented with 1 mg/L of either cefotaxime or ceftriaxone, incubated at 44°C.

Results.

Both pre-enrichment using BPW and MacConkey broth allowed the detection of bacteria producing 7 of the 8 enzymes at a detection limit of 0.1 CFU/g in meat samples and 1 CFU/g in bovine cecal samples. The same was observed for pre-enrichment in BPW of bacterial spikes in porcine cecal samples. Pre-enrichment in MacConkey broth of spiked porcine cecal samples failed to detect any of the tested bacteria (even at 1000 CFU/g). Bacteria producing OXA-48 were not detected by any of the methods. For each enzyme-producer, no obvious differences in detection limits were observed between the different pre-enrichment methods, except for the porcine cecal samples and pre-enrichment in MacConkey broth as mentioned above. Also, no obvious differences were observed in detection levels when supplementing the MacConkey agar plates with either cefotaxime or ceftriaxone. Incubation of the MacConkey plates at 44°C reduced the cecal and meat accompanying flora, facilitating the detection.

Conclusions.

Protocols to specifically select for *E. coli* ESBL/AmpC producers in meat and cecal samples from pigs and cattle were developed. These include a pre-enrichment step without antibiotics in BPW and subsequent plating on selective MacConkey agar plates containing 1 mg/L cefotaxime, followed by incubation at 44°C. This method was able to detect *E. coli*-producers of all tested enzymes except OXA-48.